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Full Length Article

Metabolites Adjustment is Crucial to Cross-Locational Adaptability of Lemongrass Populations from Quetta and Faisalabad: Evidence from Reciprocal Swap Study

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Abstract

The adaptation of plants to diverse ecological zones is challenging for plant survival. Synthesis and accumulation of metabolites could be a crucial factor in the survival of plants in assorted climates. Swapping of populations from one area to another may be a constraint for the swapped population. In this study, two lemongrass [*Cymbopogan citratus* (DC) Stapf.] populations *i.e*. native of Quetta (QN) and Faisalabad (FN) was reciprocally swapped at Quetta and Faisalabad, denoted as Quetta adapted (QA) and Faisalabad adapted (FA), and the changes in the levels of primary and secondary metabolites were measured. The data regarding metabolite changes were recorded in each month from June (Jun) to December (Dec) during the experimental years 2015 and 2016. The results revealed that primary metabolites i.e. soluble sugars, free proline, glycine betaine and total free amino acids were significantly higher during the summer months in the FA populations in comparison to FN while in Quetta a reverse trend was observed; the QA population showed a great increase in the synthesis of primary metabolites in the winter months (Oct to Dec) to overcome the prevailing chilling conditions of Quetta. Secondary metabolites data showed that soluble phenolics, anthocyanins, flavonoids, alkaloids, saponins and tannins were higher in the shoot as compared to the roots. In FN population secondary metabolite levels declined in the summer season during both experimental years while in FA population maximum secondary metabolites accumulation was noted in the summer season that eventually decreased thereafter from August (Aug) to Dec of both years. Contrarily, in QN population higher secondary metabolites contents were confirmed in the summer season. However, in QA population maximum secondary metabolite compounds were ascertained in the winter season. An increase in the synthesis of primary metabolites in adapted populations was a preferred strategy of lemongrass populations to exhibit cross-locational adaptability.

Keywords: Phytoalexins; Reciprocal swapping; Adaptability; Correlations; Lemongrass populations

Introduction

Plants show the synthesis of a larger number of compounds via secondary metabolic pathways. Kössel (1891) was the first who defined the secondary metabolites and evaluated their role in the adaptability of plants to their environments. Plant secondary metabolites have no direct role in plant growth and development, but their presence is necessary and sometimes a specific secondary metabolite is a distinguishing feature of a plant species to support its growth and development (Asten *et al.* 2019). There are two major classes of secondary compounds, which include nitrogen containing and non-nitrogen containing metabolites. Among the non-nitrogen containing secondary metabolites, the synthesis of phenolics, flavonoids, anthocyanins, and tannins have been regarded as the most important, while among the nitrogen containing secondary metabolites, alkaloids and saponins are considered important in ensuring the plant survival in changing climates (Theis and Lerdau 2003; Isah 2019).

Depending upon their biological roles, the metabolites are placed into two broad categories; primary and secondary. They exist either as phytoanticipins or phytoalexins and play their physiological roles in defense against biotic and abiotic stresses (Moradi 2016; Tiku 2020). The primary metabolites are low molecular weight compounds and are accumulated as an early response to growth limiting conditions. Secondary metabolites typically have a very limited distribution in the plant kingdom but plants spend quite a bit of energy on their production when required (Siemens *et al.* 2002). They play very important roles in plant defense, especially against herbivory and

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environmental stresses (Moradi 2016; Scott *et al.* 2020). Tolerance to harsh environmental conditions is one of the key factors in the adaptability of a plant in an area. The accumulation of the secondary metabolites in high amounts results in better resistance of plants against abiotic stresses (Mahmood *et al.* 2014; Alhaithloul *et al.* 2020).

Lemongrass [*Cymbopogan citratus* (DC) Stapf.] is an important commercial C_4 aromatic grass belonging to the family Poaceae. It is cosmopolitan in distribution, can thrive in diverse habitats ranging from the sea to mountains. It has an average life span of about 5–6 years (De Boer 2005). It propagates profusely through rhizome, and produces tillers, which add to plant biomass (Tajidin *et al.* 2012). Lemongrass is known for its lemon like aroma due to having essential oils in it (Joy *et al.* 2006). The quality and quantity of essential oils not only vary due to geographical origin and habitat but also because of agronomic practices and genetic diversity (Khanuja *et al.* 2005; Negrelle and Gomes 2007). A number of studies reported that lemongrass has antioxidant and antimicrobial roles for humans (Francisco *et al.* 2011; Mirghani *et al.* 2012). The aroma of lemongrass leaves also repels insects, especially mosquito (Joy *et al.* 2006).

Swapping the populations of a cosmopolitan plant species like lemongrass in diverse environments is a pragmatic approach to find out novel mechanisms of their adaptability and survival. In earlier studies, it is established that photosynthetic pigment composition, oxidative damage parameters and nutrient composition were closely related to the meteorological condition at both locations (Shaukat *et al.* 2018a, b). However, information is lacking regarding the associations of metabolites in modulating the growth of swapped lemongrass populations elsewhere. Varied accumulation patterns of both primary and secondary metabolites are an important manifestation of stress tolerance in plants. The data regarding swapping lemongrass populations are not reported in relation to primary and secondary metabolites synthesis and accumulation. It is predicted that the specific accumulation patterns of different metabolites during cross-adaptation make the lemongrass apt to grow in a new location. In this study, lemongrass populations growing in Faisalabad and Quetta were reciprocally swapped to determine the possible role of the biosynthesis of different metabolites in the successful survival of the swapped populations in new locations in comparison to the native counterparts.

Materials and Methods

Experimental plan

The populations used in this study were obtained from the Arid Zone Research Institute, Quetta (QN and FA) and Botanical Garden, University of Agriculture, Faisalabad (FN and QA). Field experiments were conducted to determine the cross-adaptability of lemongrass populations native to Faisalabad (FN) and Quetta (QN) in order to explore the metabolites adjustments in a reciprocal swap arrangement across the locations. The reciprocally swapped population from Quetta to Faisalabad was named as Quetta adapted (QA) and that from Faisalabad to Quetta was called as Faisalabad adapted (FA). The plants from Quetta were grown in the field in Botanical Garden (Sq. No. 32), University of Agriculture, Faisalabad. Likewise, the plants from Faisalabad were grown in fields at the Environmental Protection Agency, Quetta. Both populations were grown under field conditions at the respective locations in the month of April. The experiments were laid out in randomized complete block design (RCBD) in three replications. As given in Shaukat *et al.* (2018a, b), prior to planting of populations, the soil samples from both locations were analyzed for the following physicochemical characteristics: The AB-DTPA extractable P (mg/g) 1.56 (Qta) and 2.24 (Fsd); K (mg/g) 184 (Qta) and 162 (Fsd); nitrate-N 285 (Qta & Fsd); organic matter (%) 0.532 (Qta) and 1.10 (Fsd); saturation percentage 39.8 (Qta) and 38.6 (Fsd); pH 8.16 (Qta) and 8.0 (Fsd); electrical conductivity of extract (dS/m) 1.35 (Qta) and 0.435 (Fsd); Na^+ (mg/g) 6.84 (Qta) and 1.75 (Fsd); bicarbonate (mg/g) 2.95 (Qta) and 2.63 (Fsd); Cl- (mg/g) 5.75 (Qta) and 1.68 (Fsd); Ca+Mg (mg/g) 18.15 (Qta) and 4.18 (Fsd) and sodium adsorption ratio 2.54 (Qta) and 1.17 (Fsd). The temperature data from Qta and Fsd during the experimental periods are given in Fig. 1 (adapted from Shaukat *et al.* 2018a, b).

Tissue sampling

For tissue metabolites analysis, the plants were harvested in June (Jun), July (Jul), August (Aug), September (Sep), October (Oct), November (Nov) and December (Dec). Shoots were separated from roots. The shoots were briefly washed to remove the debris while roots were thoroughly washed to remove the adhering soil and both were blotted dry. Both shoots and roots were fractioned for fresh and dry analyses. The fraction for fresh analysis was transferred to the freezer at-40^{o}C until analyzed while for dry analysis, the plant material was dried in an oven at 65^{o}C for seven days and preserved until analyzed. The harvested whole of the shoot and root tissues in each month from different experimental units were subjected to the metabolite's analyses.

Primary metabolites analysis

Soluble sugars: To measure the soluble sugars following the method of Yoshida *et al.* (1976), (0.1 g) fresh plant material was boiled in 5 mL distilled water in water bath at 90ºC for 1 h. The extract was filtered and 1 mL of extract was diluted to 9 mL with distilled water. A 0.5 mL of the diluted extract was taken and 5 mL of anthrone reagent (prepared by dissolving 1 g anthrone (Sigma, USA) to 1 L of concentrated H_2SO_4). The mixture was briefly vortexed and placed in a water bath at 90ºC for 20 min; cooled and absorbance was taken at 620 nm on spectrophotometer.

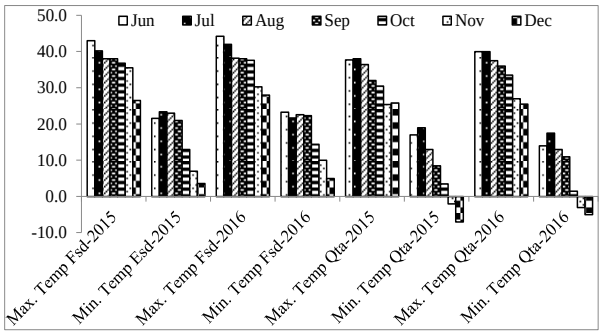

Fig. 1: Average monthly maximum and minimum temperatures (^oC) in Faisalabad and Quetta during the experimental years 2015 and 2016

Total free amino acids (TFAA): Hamilton and Van Slyke (1943) method was used to measure TFAA. Fresh plant material (0.1 g) was extracted in phosphate buffer (pH 7) and 1 mL of the extract was mixed with 1 mL of 2% ninhydrin solution and 1 mL of 10% pyridine solution. Heated the mixture for 30 min. cooled and diluted up to 25 mL. Absorbance was taken at 535 nm. Phosphate buffer was used as blank.

Free proline: The free proline was determined by using the method given by Bates *et al.* (1973). Fresh plant material (0.1 g) was homogenized in 20 mL 3% aqueous sulfo-salicylic acid and filtered. To 1 mL of the extract in a test tube, 1 mL of acid ninhydrin and 1 mL of glacial acetic acid were added. Heated the mixture in a water bath at 100^oC for 1 h and terminated the reaction in an ice bath. The reaction mixture was then extracted with 2 mL of toluene by vigorous vortexing for 15–20 sec, aspirated the colored solution and measured absorbance at 520 nm.

Glycine betaine (GB): The GB was determined by Grieve and Grattan (1983) method. A 0.5 g dried plant sample was mechanically shaken in 20 mL of distilled water for 24 h, filtered and frozen. One mL of the thawed extract was mixed with 1 mL of 2N H_2SO_4, and 0.5 mL of this solution was added in 0.2 mL potassium tri-iodide in test tube and cooled at 4^oC for 16 h. The test tubes were centrifuged at 0^oC at 10,000 prm for 15 min and aspirated the supernatant quickly with an aspiration tube. The periodide crystals in the bottom were dissolved in 9 mL of 1, 2-dichloroethane by vortexing. The tubes were let stand at 25oC for 2.5 h and measured at 365 nm.

Secondary metabolites determination

Soluble phenolics: Fresh plant material (100 mg) was ground in 1 mL of 80% acetone and centrifuged at 12000 rpm for 15 min, separated in a microfuge tube and stored at 20^oC until used. A 100 μL of supernatant diluted with distilled water to 1 mL in a 10 mL capacity test tube was added with 0.5 mL of folin phenol reagent. Shaken the sample vigorously, and added 2.5 mL of 20% Na_2CO_3. Volume was made up to 5 mL, vortexed vigorously for 5–10 sec and waited for 20 min. The absorbance was measured at 750 nm by setting spectrophotometer background to zero with 80% acetone. Standard curve was prepared using tannic acid (Julkunen-Tiitto 1985).

Anthocyanins: For anthocyanins determination by Stark and Wray (1989) method, fresh plant material (0.1 g) was extracted in 2.5 mL of acidified methanol (1% HCl, v/v); heated at 50^oC for 1 h and filtered the extract. The absorbance of mixture was taken at 535 nm. Acidified methanol was used as blank.

Flavonoids: Flavonoids were determined following the method of Zhishen *et al.* (1999). A 0.1 g fresh plant material was extracted in 80% acetone (Merck or BDH). Then added 1 mL of extract in 10 mL of volumetric flask, containing 4 mL distilled water and after 5 min, 0.6 mL of 5% $NaNO_2$ and 0.5 mL of 10% $AlCl_3$ were mixed. After 1 min, 2 mL of 1 M NaOH was also added. Diluted the reaction mixture using 2.4 mL of distilled water and absorbance was measured at 510 nm by using a spectrophotometer, while 80% acetone was used as blank.

Tannins: Fresh plant material (0.1 g) was transferred to 2 mL of diethyl ether and left for overnight. Then, decanted the solution and 1 mL of 70% acetone was added and kept for overnight. To analyze tannins, 50 μL of the extract was taken in test tube and the volume was made up to 1 mL. After dilution, 0.5 mL of Folin Phenol Reagent was added and mixed thoroughly. Then 2.5 mL of 20% Na_2CO_3 solution was mixed well and kept at room temperature for 40 min. Absorbance was taken at 725 nm using 70% acetone as blank.

Statistical analysis

The data recorded from each location for different metabolites were analyzed statistically using Statistix8.1 online software. The data means were compared using least significant difference (LSD) test at 5% probability level. Correlations of maximum and minimum temperature and shoot and root dry weight with the concentrations of primary and secondary metabolites were also established to validate their possible role in lemongrass adaptability while swapped.

Results

Statistical analysis of data revealed that in the year 2015, there was a significant difference among the months for all shoot and root parameters of native and adapted (swapped) populations except shoot flavonoids (SFLA) in Faisalabad and shoots glycine betaine (SGB) and SFLA in Quetta. Populations, on the other hand, indicated significant differences in most of the parameters except root dry weight, shoot free proline (SFP) and SGB, root soluble phenolics (RSP), SFLA, shoot anthocyanins (SANT) and root tannins (RTAN) in Faisalabad while root dry weight and root anthocyanins (RANT) in Quetta. The months ×

Table 1: Analysis of variance (mean squares) of sampling months, lemongrass populations and their interactions wheat flag leaf and grain characteristics under seed priming and foliar spray treatments at two locations in Faisalabad in the year 2015 and 2016

Parameters	2015				2016			
	Months (M) (df = 6)	Populations (P) (df = 1)	M × P (df = 6)	EMS (df = 70)	Months (M) (df = 6)	Populations (P) (df = 1)	M × P (df = 6)	EMS (df = 70)
Faisalabad								
Shoot dry weight	47702.80**	2433.80**	1902.00**	80.30	69971.50**	16.00ns	209.60*	70.60
Root dry weight	775.14**	20.20ns	14.54ns	7.56	891.74**	493.99**	50.19**	5.90
Shoot soluble sugars	71.21**	15.63**	181.67**	1.35	14.62*	84.53**	48.71**	5.47
Root soluble sugars	90.02**	8.80**	139.81**	1.16	37.61**	55.18**	91.10**	3.42
Shoot total free amino acids	1405.40**	13661.30**	4857.00**	73.00	6900.90**	224.40ns	14674.10**	492.00
Root total free amino acids	596.91**	222.39*	2972.46**	46.23	3915.30**	18540.30**	10236.80**	173.30
Shoot free proline	184.87**	0.019ns	561.53**	11.37	443.31**	1770.66**	566.82**	44.67
Root free proline	174.89**	845.68**	949.56**	5.59	413.35**	1486.68**	560.80**	20.79
Shoot glycine betaine	28.06**	0.65ns	51.59**	1.60	36.79**	15.92**	59.86**	1.23
Root glycine betaine	8.15**	271.30**	23.28**	0.42	29.08**	11.93**	39.05**	1.11
Shoot soluble phenolics	138.53**	29.82**	553.91**	10.79	245.09**	0.02ns	471.22**	16.55
Root soluble phenolics	115.02**	13.00ns	235.73**	10.76	57.07**	8.45ns	168.47**	13.21
Shoot flavonoids	0.55ns	0.03ns	17.87**	0.32	1.29**	0.02ns	8.12**	0.20
Root flavonoids	2.51**	8.29**	16.94**	0.15	1.70**	3.49**	7.70**	0.15
Shoot anthocyanins	0.09**	0.03ns	0.20**	0.003	0.04**	1.06**	0.51**	0.003
Root anthocyanins	0.11**	0.16**	0.34**	0.003	0.04**	0.56**	0.32**	0.003
Shoot tannins	1102.86**	1402.22**	2316.94**	38.84	77.63**	1990.12**	448.12**	34.83
Root tannins	193.70**	54.07ns	1313.59**	19.98	153.34**	408.68**	613.22**	51.32
Quetta								
Shoot dry weight	78.24.58**	1867.20**	42.48ns	43.87	35587.70**	69073.70**	7376.90**	95.70
Root dry weight	498.13**	10.36ns	18.03**	3.99	490.86**	496.46**	52.97**	5.98
Shoot soluble sugars	665.68**	44.43**	43.33**	3.77	50.53**	361.56**	138.71**	2.90
Root soluble sugars	128.46**	100.28**	28.26**	3.75	29.86**	181.65**	39.69**	2.45
Shoot total free amino acids	1775.11**	3216.35**	5629.63**	143.82	1970.00**	11345.00**	6375.40**	353.20
Root total free amino acids	6741.95**	429.27**	1741.64**	61.53	2518.80**	11466.60**	3673.80**	221.80
Shoot free proline	9.23**	268.78**	416.05**	11.14	186.24**	270.68**	721.34**	26.20
Root free proline	1281.57**	206.62**	399.40**	13.46	176.26**	77.92**	372.03**	12.22
Shoot glycine betaine	0.99ns	31.70**	53.62**	1.18	42.04**	520.21**	121.65**	3.24
Root glycine betaine	5.56**	19.31**	44.15**	0.66	34.05**	136.16**	135.91**	1.58
Shoot soluble phenolics	24.99**	383.14**	153.50**	12.04	156.00**	263.10**	463.00**	17.97
Root soluble phenolics	292.32**	149.48**	297.14**	5.53	105.76**	35.37*	412.47**	9.90
Shoot flavonoids	0.33ns	5.24**	10.08**	0.21	1.82**	44.06**	6.75**	0.23
Root flavonoids	0.62*	0.43*	9.18**	0.20	2.24**	5.26**	7.85**	0.19
Shoot anthocyanins	0.03*	0.07**	0.15**	0.004	0.10**	0.00ns	0.35**	0.005
Root anthocyanins	0.06**	0.01ns	0.10**	0.003	0.05**	0.08**	0.13**	0.004
Shoot tannins	9.81**	69.30**	1907.91**	32.51	205.04**	3.88ns	1396.77**	44.07
Root tannins	685.17**	195.43**	1193.92**	27.21	285.34**	272.16**	954.37**	31.81

df, degree of freedom
EMS, Error mean square
Significant at: *, P<0.05; **, P<0.01 and ns, P>0.05

populations interaction was significant for all parameters except root dry weight in Faisalabad and RANT in Quetta (Table 1). In 2016, months showed significant differences for all the parameters at both the locations. As for populations most of the parameters of exhibited significant differences at both locations excepting shoot total free amino acids (STFAA), SSP and RSP and SFLA in Faisalabad while SANT and STAN in Quetta. The months × populations interactions were also significant for all the growth and metabolite attributes (Table 1).

Plant biomass

Irrespective of the populations, the dry mass of shoot and root increased with the plant age. At both the locations and in all swapped populations, the both the shoot and root dry weight was relatively lower in 2015 as compared to 2016. FN population displayed the highest shoot and root dry mass followed by FA while these parts exhibited the lowest dry mass in QN (Fig. 2). This may be assigned to relatively more adverse prevailing temperature in the years 2015.

Primary metabolites accumulation pattern

Overall, the levels of primary metabolites in the shoot (SSS, STFAA, SFP and SGB) were higher than the root (RSS, RTFAA, RFP and RGB) in both the years (Fig. 3). The SSS and RSS contents were higher in FN and QA population as compared to QN and FA populations. In FN population highest SSS and RSS contents were observed from Nov to Dec. However, in FA population maximum SSS contents were detected in the months of Jun and Jul that gradually decreased thereafter. As far as QN (Quetta native) population was concerned SSS content increased from Jun to Aug. Furthermore, in QA population higher SSS and RSS contents were ascertained in the month of Dec (Fig. 3).

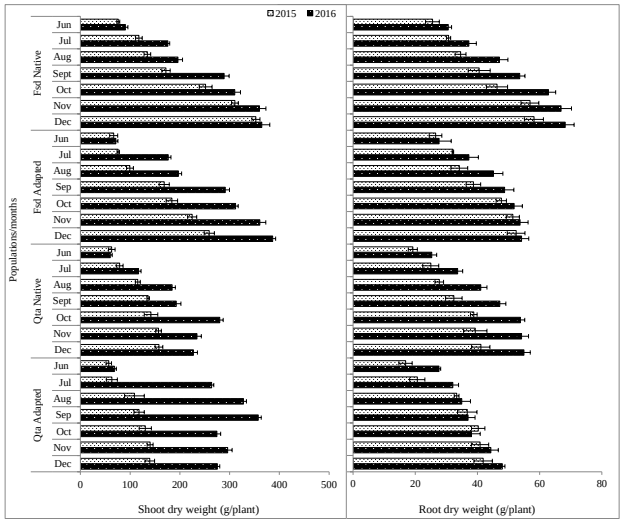

Fig. 2: Monthly changes in the shoot and root dry weight in native and reciprocally swapped and adapted lemongrass populations grown in Faisalabad and Quetta during 2015 and 2016

Considering STFAA and RTFAA and SFP and RFP, it was observed that both these osmoprotectants were significantly higher during Oct to Dec in FN population while in FA population subsequently decreased from Jul to Dec. On the other hand, in QN population, maximum STFAA and SFP content was detected during Jul and Aug followed by RTFAA and RFP. Conversely, in QA population STFAA and SFP content was higher in the winter season followed by RTFAA and RFP (Fig. 3). Higher SGB and RGB content was observed in the winter season in FN population, while in FA population maximum SGB and RGB was detected in the summer season during 2015 and 2016. Conversely, in QN population higher SGB contents followed by RGB were observed in months of Jun to Aug; however, in QA population higher SGB followed by RGB contents were detected in the month of Dec. On the other hand, in QN population higher SGB and RGB contents were analyzed in the summer season. In QA population higher SGB and RGB content was confirmed in the winter season (Fig. 3).

Secondary metabolites accumulation pattern

Data regarding secondary metabolites in shoot (SSP, SFLA, SANT, STAN) and root (RSP, RFLA, RANT, RTAN) revealed that, with few exceptions, SSP and RSP indicated similar trend of accumulation in FN and QA populations being higher during Oct-Dec in both the years, while in FA and QN populations their accumulation was greater during Jul-Sep (Fig. 4). In FN and QA populations, the shoot and root levels of flavonoids kept low Jun–Sep but began to accumulate later and attained the highest level in Dec. However, in FA and QN populations, the SFLA and RFLA contents were higher in Aug–Sep of both the years (Fig. 4). The SANT accumulation in FN population was quite exaggerated but such a trend was not seen in 2016 in FN and 2015 and 2016 when the anthocyanins declined from Jun–Sep. On the other hand, in FA and QN populations, the accumulation of SANT and RANT was the highest during Jul–Sep in both the experimental years (Fig. 4). In the STAN and RTAN of FN and QA populations during both the years declined from Jun–Oct but depicted a substantial gain from Oct–Dec. Contrarily, FA and QN lemongrass populations indicated much higher levels of STAN and RTAN from Jul–Aug in 2015 than 2016 (Fig. 4). Overall, the results revealed that the accumulation of the studied secondary metabolites was relatively higher in the shoot than in the root tissue, except for flavonoids, which were comparable in both these tissues (Fig. 4).

Correlations

Metabolites association with maximum and minimum temperature: In view of the fact that prevailing temperature is a major factor affecting the growth and metabolism of lemongrass populations, the correlations of the levels of primary and secondary metabolites were established with maximum and minimum temperatures based on their changes during Jun–Dec of 2015 and 2016 in the native (FN, QN) and swapped (FA, QA) populations (Table 2). Among the primary metabolites, shoot soluble sugars (SSS) in FN population were negatively correlated with maximum and minimum temperature in both the years; FA population showed positive correlation with minimum temperature in both the years; QN population exhibited a positive correlation with minimum temperature during 2016 while QA population indicated negative correlation with both maximum and minimum temperatures in both the years for SSS. For RSS, FN population indicated negative correlation with maximum and minimum temperatures in 2016 only, while for FA population, a positive correlation was noted with maximum temperatures in 2015 only and with both the temperatures in 2016. QN showed no association with any temperatures in both the years while QA indicated negative correlation with maximum and minimum temperatures in 2015 only for RSS. For STFAA, FN population revealed negative correlation with maximum and minimum temperatures in 2015 only, while FA population showed positive correlation with both the temperatures in both the years except with maximum temperature in 2015. QN exhibited positive correlation with maximum and minimum temperatures in 2016 only while QA indicated negative correlation of both the temperatures in 2015 only for STFAA. RTFAA in FN population was negatively correlated with maximum and minimum temperatures in 2015; while for FA this attribute was positively correlated with both the temperatures in both the years excepting no correlation of maximum temperature in 2015. In QN population, the RTFAA was positively correlated with both the temperatures in 2016 while no correlation of this attribute was noted with temperatures in

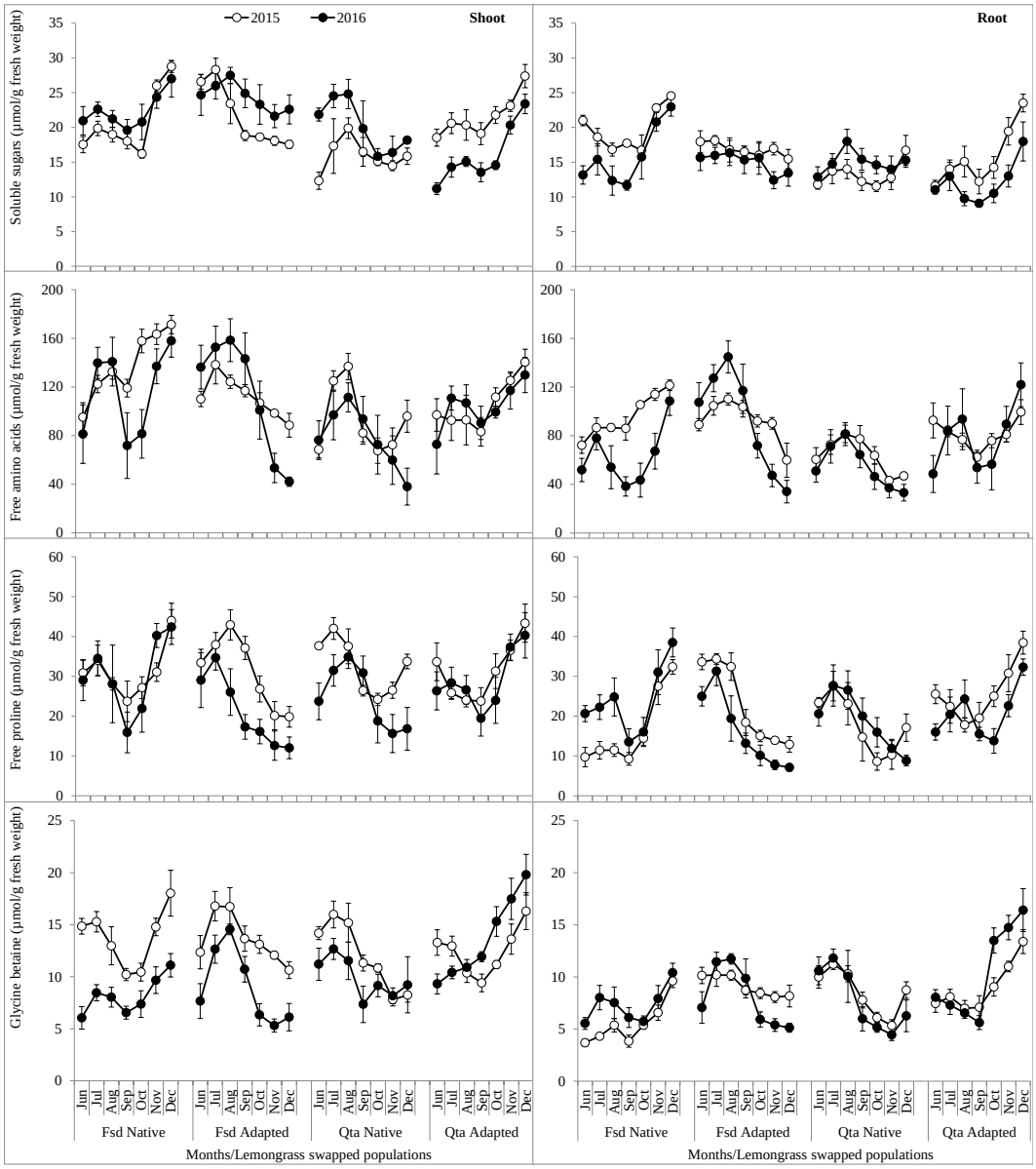

Fig. 3: Monthly changes in the shoot and root levels of primary metabolites in native and reciprocally swapped and adapted lemongrass populations grown in Faisalabad and Quetta during 2015 and 2016

both the years in QA population (Table 2).

The SFP accumulation was not correlated with temperatures and years in FN population while in FA positive correlations of maximum and minimum temperatures were noted with SFP in both the years except with maximum temperature in 2015. QN indicated positive correlation of SFP with both the temperatures in 2015 while QA manifested negative correlation of SFP with both the temperatures in 2016. For RFP, the FN population indicated negative correlation with both the temperatures in 2015 only, while FA indicated positive correlation with both the temperatures in both the years except with maximum temperature in 2015. QN showed positive correlation with both the temperatures in 2016 only, whereas QA indicated only negative correlation minimum temperature with RFP in 2015 only. Data revealed that SGB in FN population was negatively correlated with maximum and minimum temperature in 2016; was not correlated with both the temperatures in 2015 or 2016 in FA population; was positively correlated with both the temperatures in QN population in 2015 only, while QA population was negatively correlated with maximum and minimum temperatures in QA population in 2016. The RGB was negatively correlated with both the temperatures in FN population in 2015; was positively correlated with minimum temperature in FA population in 2015 and 2016; was positively correlated with both the temperatures and years in QN population with the exception of minimum temperature

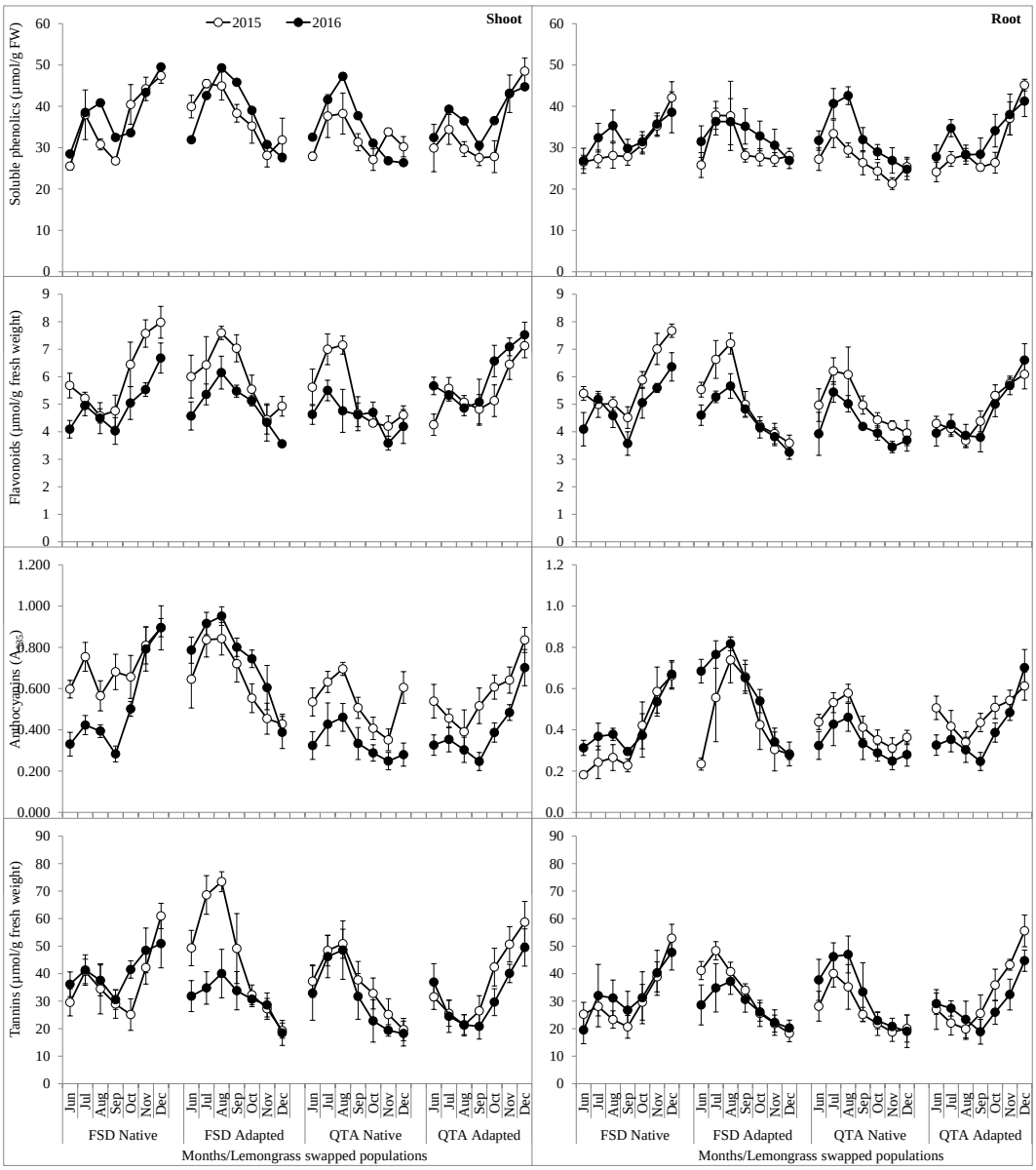

Fig. 4: Monthly changes in the shoot and root levels of secondary metabolites in native and reciprocally swapped and adapted lemongrass populations growing in Faisalabad and Quetta during 2015 and 2016

in 2015 but negatively correlated with both the temperatures and years in QA population except minimum temperature in 2016 for this attribute (Table 2).

As regards secondary metabolites, SSP in FN population revealed negative correlation with maximum and minimum temperatures in both the years except no correlation of minimum temperature with this attribute. FA population revealed no correlation of SSP with maximum or minimum temperature in 2015 and 2016 except significant correlation of minimum temperature with SSP in 2015. SSP in QN population indicated no correlation with both maximum and minimum temperatures in both the years except a positive correlation of minimum temperature, while QA population indicated no relationship with SSP maximum and minimum temperatures in both the years. The RSP of FN population indicated negative correlation of both the maximum and minimum temperature in both the years except no relationship with minimum temperature in 2016, while reverse of it was true for FA population. QN population indicated positive while QA population showed negative correlation with maximum and minimum temperatures in 2015 as well as 2016. The SFLA in FN population exhibited negative correlation with maximum and minimum temperatures while FA population revealed positive correlation of this attribute with minimum temperature in both the years. QN population indicated positive while QA population showed negative correlation with maximum and minimum temperatures in 2015 as well

Table 2: Correlation of changes in secondary metabolites in the shoot and root tissues of lemongrass populations over different sampling months during 2015 and 2016 (Faisalabad and Quetta) with maximum and minimum temperatures (n = 7)

Parameter	Lemongrass population	2015		2016	
		Max Temp	Min Temp	Max Temp	Min Temp
Shoot soluble sugars	Fsd Native	-0.802*	-0.789*	-0.772*	-0.843*
	Fsd Adapted	0.717ns	0.765*	0.678ns	0.823*
	Qta Native	0.563ns	0.479ns	0.750ns	0.858*
	Qta Adapted	-0.780*	-0.849*	-0.928**	-0.833*
Root soluble sugars	Fsd Native	-0.579ns	-0.725ns	-0.838*	-0.952**
	Fsd Adapted	0.826*	0.683ns	0.845*	0.831*
	Qta Native	0.312ns	0.422ns	0.034ns	0.128ns
	Qta Adapted	-0.783*	-0.834*	-0.666ns	-0.589ns
Shoot total free amino acids	Fsd Native	-0.841*	-0.872**	-0.543ns	-0.484ns
	Fsd Adapted	0.699ns	0.891**	0.879**	0.972**
	Qta Native	0.445ns	0.377	0.821*	0.854*
	Qta Adapted	-0.816*	-0.864*	-0.717ns	-0.598ns
Root total free amino acids	Fsd Native	-0.883**	-0.940**	-0.578ns	-0.665ns
	Fsd Adapted	0.772ns	0.825*	0.801*	0.947**
	Qta Native	0.749ns	0.723ns	0.786*	0.853*
	Qta Adapted	0.116ns	-0.212ns	-0.610ns	-0.464ns
Shoot free proline	Fsd Native	-0.672ns	-0.580ns	-0.554ns	-0.644ns
	Fsd Adapted	0.670ns	0.958**	0.850*	0.783*
	Qta Native	0.690ns	0.625ns	0.780*	0.871*
	Qta Adapted	-0.687ns	-0.734ns	-0.760*	-0.675ns
Root free proline	Fsd Native	-0.866*	-0.947**	-0.741ns	0.745ns
	Fsd Adapted	0.729ns	0.848*	0.843*	0.768*
	Qta Native	0.792ns	0.742ns	0.911**	0.949**
	Qta Adapted	0.735ns	-0.772*	-0.597ns	-0.447ns
Shoot glycine betaine	Fsd Native	-0.440ns	-0.421ns	-0.847*	-0.838*
	Fsd Adapted	0.574ns	0.836*	0.523ns	0.735ns
	Qta Native	0.955**	0.957**	0.630ns	0.643ns
	Qta Adapted	-0.418ns	-0.462ns	-0.980**	-0.973**
Root glycine betaine	Fsd Native	-0.956**	-0.871*	-0.716ns	-0.669ns
	Fsd Adapted	0.713ns	0.866*	0.584ns	0.786*
	Qta Native	0.787*	0.703ns	0.790*	0.840*
	Qta Adapted	-0.831*	-0.866*	-0.884**	-0.938**
Shoot soluble phenolics	Fsd Native	-0.763*	-0.841*	-0.832*	-0.745ns
	Fsd Adapted	0.610ns	0.912**	0.494ns	0.720ns
	Qta Native	0.308ns	0.339ns	0.747ns	0.818*
	Qta Adapted	-0.686ns	-0.698ns	-0.745ns	-0.669ns
Root soluble phenolics	Fsd Native	-0.950**	-0.894**	-0.839*	-0.730ns
	Fsd Adapted	0.197ns	0.537ns	0.681ns	0.834*
	Qta Native	0.828*	0.783*	0.774*	0.843*
	Qta Adapted	-0.780*	-0.826*	-0.816*	-0.773*
Shoot flavonoids	Fsd Native	-0.755*	-0.963**	-0.833*	-0.935**
	Fsd Adapted	0.508ns	0.872*	0.601ns	0.790*
	Qta Native	0.820*	0.770*	0.811*	0.800*
	Qta Adapted	-0.765*	-0.779*	-0.884**	-0.925**
Root flavonoids	Fsd Native	-0.975*	-0.955**	-0.756*	-0.863*
	Fsd Adapted	0.639ns	0.882**	0.746ns	0.902**
	Qta Native	0.861*	0.861*	0.761*	0.790*
	Qta Adapted	-0.921**	-0.919**	-0.922**	-0.910**
Shoot anthocyanins	Fsd Native	-0.774*	-0.771*	-0.901**	-0.965**
	Fsd Adapted	0.757*	0.941**	0.827*	0.919**
	Qta Native	0.572ns	0.445ns	0.763*	0.806*
	Qta Adapted	-0.822*	-0.864*	-0.838*	-0.803*
Root anthocyanins	Fsd Native	-0.878**	-0.978**	-0.898**	0.928**
	Fsd Adapted	0.250ns	0.628ns	0.872*	0.952**
	Qta Native	0.838*	0.780*	0.723ns	0.806*
	Qta Adapted	-0.734ns	-0.744ns	-0.838*	-0.803*
Shoot tannins	Fsd Native	-0.706ns	-0.636ns	-0.755*	-0.892**
	Fsd Adapted	0.680ns	0.924**	0.714ns	0.875**
	Qta Native	0.891**	0.889**	0.824*	0.898**
	Qta Adapted	-0.882**	-0.900**	-0.731ns	-0.741ns
Root tannins	Fsd Native	-0.873*	-0.904**	-0.916**	-0.891**
	Fsd Adapted	0.800*	0.946**	0.761*	0.876**
	Qta Native	0.879**	0.862*	0.878*	0.945**
	Qta Adapted	-0.889**	-0.923**	-0.704ns	-0.670ns

Significant at: *, P<0.05; **, P<0.01 and ns, P>0.05

as 2016. AS regards RFLA, FN population indicated negative correlation with both the temperatures in both the years while FN population showed positive correlation with minimum temperature only in both the years. The QN population manifested positive while QA population indicated negative correlation with temperatures in the years for RFLA (Table 2).

As for SANT, FN population indicated negative while FA showed positive relationship with maximum and minimum temperature in both the years. FA population indicated positive correlation for SANT in 2016 only while QA population exhibited negative relationship with this attribute for both the temperatures and years. The RANT of FN population showed negative correlation with both temperatures and years while FA population showed positive correlation with both temperatures with this character in 2016 only. Likewise, RANT of QN population showed positive correlation with both the temperatures in both the years while RANT of QA population exhibited negative correlation with maximum and minimum temperature in 2016 only. As regards STAN, there was no correlation of this attribute with maximum and minimum temperatures in 2015 but significant ones with both the temperatures in 2016. The FA population indicated positive correlations of STAN with minimum temperatures in both the years. The STAN of QN population manifested positive correlation with both maximum and minimum temperatures in both the years while QA population showed a negative correlation of this attribute with both the temperatures in 2015 only. As for RTAN, both maximum and minimum temperatures showed negative correlations for FN population but positive for FA and QN population in both the years while for QA population, negative correlations of maximum and minimum temperatures were noted in the year 2015 only (Table 2).

Metabolites association with plant biomass: The correlations were established to substantiate the role of metabolites accumulation in the dry biomass production of the respective parts in both the years (Table 3). As regards SSS and RSS contents, FN population indicated positive and FA showed negative correlation with shoot dry weight while FA population revealed negative correlation for root dry weight. However, QN and QA populations showed no correlation with shoot or root dry weight with SSS or RSS respectively in 2015. On the other hand in 2016, both shoot and root dry weight held no association with SSS and RSS. FN and FA populations indicated positive and negative correlations, respectively of shoot dry weight with STFAA while FN indicated positive correlation with RTFAA in 2015 and STFAA with shoot dry weight in 2016. However, QN and QA manifested no correlation of STFAA and RTFAA with shoot and root dry weight in 2016. SFP revealed no correlation with shoot dry weight of FN, QN and QA populations but showed a negative correlation with this attribute of QN in 2015. RFP of FN population indicated positive while those of FA and QN populations indicated negative correlations while QA revealed no association with root dry weight in 2015. However, in 2016 except for a negative correlation of shoot and RFP of FA, none of the population indicated any correlation with RFP of the respective parts. SGB showed no correlation with

Table 3: Correlation of changes in shoot and root secondary metabolites with shoot and root dry weight (n = 7) of lemongrass populations over different sampling months during 2015 to 2016 (Quetta & Faisalabad)

Parameter	Lemongrass	Shoot dry weight		Root dry weight	
	Population	2015	2016	2015	2016
Soluble sugars	Fsd Native	0.754*	0.517ns	0.544ns	0.687ns
	Fsd Adapted	-0.930**	-0.675ns	-0.785*	-0.582ns
	Qta Native	-0.270ns	-0.727ns	-0.314ns	-0.220ns
	Qta Adapted	0.657ns	0.362ns	0.623ns	0.639ns
Total free amino acids	Fsd Native	0.945**	0.259ns	0.964**	0.286ns
	Fsd Adapted	-0.817*	-0.759*	-0.594ns	-0.604ns
	Qta Native	-0.236ns	-0.366ns	-0.539ns	-0.521ns
	Qta Adapted	0.619ns	0.546ns	0.222ns	0.0679ns
Free proline	Fsd Native	0.480ns	0.241ns	0.885**	0.433ns
	Fsd Adapted	-0.827*	-0.892**	-0.937**	-0.876**
	Qta Native	-0.747ns	-0.467ns	-0.810*	-0.721ns
	Qta Adapted	0.392ns	0.044ns	0.384ns	0.641ns
Glycine betaine	Fsd Native	0.275ns	0.651ns	0.823*	0.439ns
	Fsd Adapted	-0.746ns	-0.494ns	-0.938**	-0.392ns
	Qta Native	-0.858*	-0.640ns	-0.760*	-0.893**
	Qta Adapted	0.071ns	0.324ns	0.573ns	0.794*
Soluble phenolics	Fsd Native	0.857*	0.617ns	0.891**	0.677ns
	Fsd Adapted	-0.878**	-0.275ns	-0.394ns	-0.335ns
	Qta Native	-0.148ns	-0.363ns	-0.694ns	-0.596ns
	Qta Adapted	0.428ns	0.181ns	0.581ns	0.807*
Flavonoids	Fsd Native	0.867*	0.685ns	0.821*	0.584ns
	Fsd Adapted	-0.677ns	-0.401ns	-0.822*	-0.530ns
	Qta Native	-0.691ns	-0.468ns	-0.725ns	-0.516ns
	Qta Adapted	0.613ns	0.062ns	0.670ns	0.874**
Anthocyanins	Fsd Native	0.785*	0.732ns	0.948**	0.698ns
	Fsd Adapted	-0.833*	-0.678ns	-0.320ns	-0.663ns
	Qta Native	-0.436ns	-0.453ns	-0.713ns	-0.564ns
	Qta Adapted	0.581ns	0.001ns	0.367ns	0.794*
Tannins	Fsd Native	0.607ns	0.577ns	0.875**	0.784*
	Fsd Adapted	-0.869*	-0.581ns	-0.938**	-0.515ns
	Qta Native	-0.668ns	-0.574ns	-0.754*	-0.785*
	Qta Adapted	0.650ns	0.373ns	0.636ns	0.623ns

Significant at: *, P<0.05; **, P<0.01 and ns, P>0.05

shoot dry weight of FN, QN and QA populations but displayed a negative correlation with this parameter of QN in 2015. Root dry weight, on the other hand, showed positive correlation with RGB in FN, negative correlation in FA and QN while no correlation with QA in 2015. In the year 2016, SGB was not correlated with shoot dry weight of any population. RGB indicated no correlation with root dry weight of FN and FA populations while it was positive correlated with dry weight of QN and negatively with QA populations in 2016 (Table 3).

Among the plant secondary metabolites, SSP of FN population indicated positive correlation, FA population showed negative correlation whereas QN and QA populations indicated no correlation with shoot dry weight in 2015. RSP of FN population indicated positive correlation while those of FA, QN and QA indicated no correlations with root dry weight in 2015. In the year 2016, SSP and RSP of none of the populations indicated any correlation with shoot and root dry weight except a positive correlation of RSP with root dry weight. In 2015, among the population SFLA of FA population only indicated positive correlation with shoot dry weight. However, for RFLA, the FN population showed positive and FN population showed negative correlation with root dry weight whereas QN and QA populations showed no correlation. In 2016, SFLA and RFLA of none of the populations indicated no associations with shoot and root dry weight except a positive correlation of RFLA with root dry weight. For SANT, FN and FA indicated positive and negative correlation with shoot dry weight in 2015 while RFLA indicated a positive correlation with root dry weight in FN only in 2015. However, in 2016, SANT and RANT of any populations indicated no association with shoot and root dry weight except a positive correlation of RANT with root dry weight. The STAN of FA population exhibited negative while that of QN showed positive correlation with shoot dry weight in 2015. The RTAN indicated positive correlation with root dry weight while rest of the populations indicated no relationship of RTAN with this attribute in 2015. However, in the year 2016, although shoot dry weight was not correlated with STAN of any population, the RTAN was positively correlated with root dry weight of FN and negatively with that of QN (Table 3).

Discussion

The prevailing temperature of an area majorly determines the success of any species; the species with inherent ability to synthesize the stress-resistance compounds are on an advantage (Raza *et al.* 2019). The statistical analysis of two years data from all populations from both the locations revealed that although the months × populations interactions were significant for all the parameters, the behavior of lemongrass populations at Faisalabad was relatively less-specific than at Quetta. Lemongrass is a C_4 tropical plant species and needs a relatively higher optimal temperature for growth. A relatively higher shoot and root dry mass of the lemongrass populations (with C4 photosynthetic pathway) growing in Faisalabad than in Quetta can be assigned to a more favorable sub-tropical condition of Faisalabad (Fig. 2). A more specific behavior of the accumulation of metabolites in lemongrass populations in Faisalabad and Quetta can be attributed to differences in the temperate semi-arid type climate of Quetta and sub-tropical climate of Faisalabad.

De novo synthesis of primary and secondary phytochemicals is important enabling the plants to respond successfully to varied environmental conditions (Murakeözy *et al.* 2003; Wahid 2007; Moradi 2016). The major biological roles of primary metabolites are to act as osmoprotectants and maintain the cytoplasmic water balance to sustain cell life, which is pivotal to withstand suboptimal conditions (Papageorgiou and Murata 1995; Slama *et al.* 2015). Their role is perceivable since under adverse conditions, the ensured availability of water is pre-requisite for hydration and sustained functioning of cytoplasmic and organelle membranes (Slama *et al.* 2015). Among the major osmoprotectants, low molecular weight sugars are accumulated in a major bulk, while the accumulation of FP and GB in the shoot and root specifically takes place under the conditions of drought, salinity and high temperature (Wahid 2007; Nahar *et al.*

2016). The results of the current study on the shoot and root accumulation of soluble sugars, total free amino acids, free proline, and glycinebetaine revealed that native Quetta population adapted in Faisalabad and Faisalabad population adapted in Quetta displayed a greater accumulation of all these primary metabolites in both shoot and root of lemongrass (Fig. 3) when the temperature was high enough in Faisalabad and chilling to freezing in Quetta (Fig. 1). The trend was similar in both experimental years with some exceptional fluctuations. These results, therefore, clearly showed that, as already reported, enhanced free proline and glycine betaine accumulation help the plants to withstand under environmental adversaries such as water stress (Yamada *et al.* 2005), heat stress (Wahid 2007) and heavy metal stress (Roy and Bera 2003). The lemongrass was able to sustain growth under prevailing sub- or supra-optimal conditions with the adjustment of primary metabolites which acted as cytosolutes in shoot and root. Furthermore, the rate of primary metabolites accumulation was similar during both the years.

Critical perusal of the results revealed that the levels of the primary metabolites were especially higher in the shoot and lesser in the root. This substantiated their osmoprotective role in the sustained growth of all populations under relatively suboptimal conditions (Hare *et al.* 1998; Chalker-Scott 1999; Wahid 2007). Under heat stress, manifold increase in free proline and soluble sugars contents was reported in *Cicer arietinum* (Khetarpal *et al.* 2009; Arunkumar *et al.* 2012). Smallwood and Bowles (2002) reported that during cold acclimation, primary metabolites such as proline and sugars accumulate in high amounts in different plants. It is also important to notice that, although in lower amounts, the accumulation of all these primary metabolites was observed in the root from the native or adapted populations in both locations (Fig. 3). This indicated that after the perception of the stress signal, the roots also synthesized such important primary metabolite, which sustained the root functions concerned with the absorption of water and nutrients. The stress induced modulations in the root temperature are considered of great significance in stress tolerance (Koevoets *et al.* 2016). The trend of accumulation of primary metabolites revealed that irrespective of their types, their levels declined when the favorable conditions prevailed. This indicated that their accumulation is only prompted once the cell perceives signals regarding a change in the ambient environment (Ramakrishna and Ravishankar 2011; Shaukat *et al.* 2018). As reported above, the production of reactive oxygen species (ROS) is the initial cellular response, which damages the cellular membranes (Wang *et al.* 2006; Königshofer *et al.* 2008; Shaukat *et al.* 2018a). So, protection from ROS damage is an important cellular strategy to withstand stress conditions.

In the current experiments, it was noted that during the summer months in Faisalabad and in the winter months in Quetta, the concentration of studied secondary metabolites was higher, which indicated their specific role in tolerance to adverse conditions of high temperature (Faisalabad) and chilling (Quetta) in the adapted and non-adapted populations (Fig. 4). Increased phenolics accumulation during the months of Jul to Sep and reduced during May was reported in *Camellia sinensis* (Anesini *et al.* 2008). However, Garmesh (2005) reported that chilling stress and plant maturity increased the concentration of flavonoids and phenolics during the winter months in barley (*Hordeum vulgare* L.). A highest accumulation of phenolics was observed during Nov in *Glycyrriza glabra* (Aires *et al.* 2011). Anthocyanin and phenolic content in blueberries (*Cyanococcus* sp.) were found to be significantly affected with maturity; however, different locations did not affect its accumulation (Prior *et al.* 1998), as also noted here. Anthocyanins entail an essential role in the adaptability of plants to environmental stresses by acting as UV screen and having an osmoregulatory role (Chalker-Scott 1999; Wahid 2007).

The secondary metabolites have more of the defensive roles against environmental perturbations by acting as phytoalexins (Moradi 2016; Yang *et al.* 2018). The accumulation of secondary metabolites with the incidence of stress conditions is a slow adaptive strategy of lemongrass, which appeared to act as a second line of defense to a new location. The results revealed that there was a greater accumulation of soluble phenolics, anthocyanins, and flavonoids, while tannins were accumulated to a lesser extent, both in the shoot and root (Fig. 4). The compounds except tannins are usually found in the soluble phase; therefore, an increase in their concentration under more adverse climatic conditions is known (Wahid 2007; Tiku 2020). Soluble phenolics act as non-enzymatic antioxidant due to having a phenol ring in their structure, which confers on them important physiological properties (Van Sumere 1989). Anthocyanins act as UV-screens when they accumulate in the epidermal cells and protect the underlying more physiologically important mesophyll tissues from the damaging effects of harmful, especially UV, radiations (Chalker-Scott 1999; Moradi 2016). Likewise, flavonoids are also soluble in nature and act as antioxidants (Agati *et al.* 2007) and protect the cytoplasmic membranes from the adverse effects of stressful conditions (Winkel-Shirley 2002). Tannins are of two types; condensed and hydrolysable. The condensed tannins are not much important physiologically because of being complex and insoluble, but hydrolysable tannins play an important role in the plant growth and development under adverse conditions (Tiku 2020). In this study we noted that the accumulation of tannins was greater during the summer months in the FA lemongrass populations and i the winter season in QA population (Fig. 4). These findings again speak of the role of these metabolites in tolerance to relatively sub-optimal environmental conditions.

The establishment of correlations of maximum and minimum temperatures with the shoot and root

accumulation of primary (soluble sugars, total free amino acids, free proline and glycine betaine) and secondary (soluble phenolics, flavonoids, anthocyanins and tannins) metabolites was specific to locations. Here the secondary metabolites were relatively more closely associated to the minimum and maximum temperature especially in QN and FA populations suggesting their perceived defense role in abiotic stress tolerance (Chalker-Scott 1999; Wahid 2007; Isah 2019) by acting as phytoalexins (Yang *et al.* 2018). These findings further revealed that the swapping had little effect on changing the inherent tendency of the populations to accumulate metabolites in response to temperature fluctuations, although the swapped populations tended to show the similar metabolites accumulation patterns to their native counterparts (Table 2).

In addition to the specific accumulation pattern of metabolites in response to prevailing temperatures, significant correlations of metabolites levels were detected with shoot and root dry weight of native and swapped populations (Table 3). The role of metabolites accumulation in improved growth and performance of plants by improving water status and reduced ROS production under relatively subversive conditions has been documented (Arbona *et al.* 2013; Isah 2019). As the results revealed, secondary metabolites showed tighter associations than the primary ones thus substantiating their greater role as defense arsenal in enabling the native and swapped (adapted) populations in their original or new locations, although the swapped populations behaved alike their native counterparts.

Conclusion

As revealed from their correlation drawn with maximum and minimum temperatures and shoot and root dry weight, the roles of both the primary and secondary metabolites were devoted in adapting the swapped population to new locations mainly by acting as phytoalexins. Primary metabolites played a major role in adjusting the swapped populations to a new environment, primarily by improved cellular water balance, which is pivotal under all conditions. The changes in secondary metabolites were not much different during both the years. They indicated delayed but consistent accumulation although their concentration varied greatly from metabolite to metabolite and population to population. Nonetheless, the secondary metabolites improved tolerance to suboptimal conditions and appeared to support later growth of the respective lemongrass populations adapting to new locations for a longer time.

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Author Contributions

KS and AW designed the experiment; NZ helped in data analysis and preparation of initial draft and SMAB finalized the script for submission

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